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STORAGE OF BIOLOGICAL SAMPLES

by T. M. Fraser

Prepared by

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH

Albuquerque, New Mexico

for

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STORAGE OF BIOLOGICAL SAMPLES

By T. M. Fraser, M.Sc., M.D.

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PREFACE

While references are included in this study for all technical papers discussed, and for some personal communications, much of the information detailed came from contact with specialists and authorities in various clinical, physiological, biochemical, and pathological fields. These included:

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STORAGE OF BIOLOGICAL SAMPLES

In clinical practice it is the common procedure to obtain a biological sample and subject it to the required measure or analysis within a few minutes or hours of its acquisition. Occasionally, suitable specimens may be stored for a more prolonged period, along with similarly stored reference materials, for later examination. Of late, it has also become common practice to submit suitably preserved or packaged samples to a distant laboratory, allowing for several days of elapsed time between acquiring and examining the samples. Methods have also been evolved for the storage of whole blood and blood cells for very prolonged periods for transfusion purposes, but until now it has not been necessary to consider the prolonged storage of biological samples for widely varying test purposes. Since there has been no requirement, little information resulting from direct experiment is available on the duration for which biological samples can be stored and still retain their stability. With the development of the requirement to analyse biological samples taken during orbital flight, it has become necessary to examine the possibilities of storage of these samples for prolonged periods.

In conducting this study, certain terms of reference are applied. It is assumed that the spacecraft will be of the Apollo type with a crew that will include at least one scientific member trained in the necessary sampling and storage techniques. The mission will be of 15, 30, 45, or 90 days duration. The 90-day mission will have a re-supply capacity with return of stored samples. All specimens suitable for storage will be so stored, and the minimum of in-flight analysis will be made. Simple storage techniques of minimal bulk and weight have priority over complex techniques. As directed, the biological samples required will be those detailed in the Lockheed study entitled Biological Measurement of Man in Space, Vols. I-VI, Lockheed Missiles and Space Company, M-61-64-1, 1965.

The various test materials and sources are shown in the following Table.

TABLE I

COMPOSITE LIST

Parameters	Serum or Plasma	Urine	Whole Blood	Feces	Sweat
Creatine	X	X			
Creatinine	X	X			
Serum Proteins (electrophoresis)	X				
Mucoproteins & related Biocolloids	X	X			
Sodium	X	X		X	X
Potassium	X	X		X	X
Chlorides	X	X		X	X
Phosphates	X				
Alkaline Phosphatase	X				
Calcium	X	X		X	X
Magnesium	X	X			
Manganese	X	X			
Bicarbonate	X				
Zinc	X	X			
Sulfates	X	X			
Pyrophosphates		X			
NPN	X				
BUN	X				
Uric acid	X				
Glucose tolerance	X				
Fat tolerance	X			X	
Amino nitrogen	X				

Parameters	Serum or Plasma	Urine	Whole Blood	Feces	Sweat
Total nitrogen	X	X		X	X
Blood lactic acid			X		
Bilirubin	X				
Standard Clinical analysis		X			
Protein Bound Iodine (PBI)	X				
17-hydroxy corticosteroids		X			
Catecholamines	X				
Thyroxine	X				
Thyroxine Binding Prealbumin (TBPA)	X				
Aldosterone		X			
Antidiuretic Hormone (ADH)	X	X			
Adreno-corticotrophic Hormone (ACTH)	X				
Serotonin		X			
Specific Gravity		X			
Proteins		X			
pH		X			
Hematocrit			X		
Reticulocyte count			X		
RBC (total)			X		
WBC (total)			X		
WBC differential			X		
RBC cell mass (isotopes)			X		
RBC survival			X		
Hemoglobin			X		
Platelet count			X		
Plasma Volume (RISA ¹²⁵)	X				

Parameters	Serum or Plasma	Urine	Whole Blood	Feces	Sweat
WBC differential			X		
WBC motility and phagocytic Activity			X		
Platelet Estimate			X		
Platelet Adhesiveness			X		
Fibrinogen	X				
Fibrinolytic Activity	X				
Prothrombin Activity	X				
Plasma Thromboplastic Component (PTC)	X				
Antihemophylic Globulin (AHG)	X				
Immunoglobulins (compliment and antibodies)	X				
Cytogenic Studies of Lymphocytes (Karyotyping)			X		
Clotting time			X		
Clot Retraction			X		

Nature of Samples

Samples to be stored comprise various body fluids and effluent, including urine, feces, blood and sweat, and because of different analytic requirements, various components of the materials may be required. Thus, urine and feces may be stored in their entirety. With respect to blood, however, consideration has to be given to the separate requirements of tests involving blood cells, serum, and plasma. Almost all the blood biochemistry demanded, and the immunological studies, can, in fact, be carried out on the serum. Standard values for serum differ on occasion from those of whole blood or plasma, (e.g., glucose content), but good reference values are available for all the requirements (Henry, 1964)⁷. Since serum is simpler to store than whole blood, and occupies less bulk than plasma, its use should be encouraged.

Sample collection, however, will entail the use of a spaceborne laboratory centrifuge to spin down the formed clot from the serum.

For many of the hematological studies, whole blood is required, while for studies in clotting dynamics and measures of blood volume, storage of plasma is required.

Samples of microbiological flora of nose, mouth, throat, and skin, etc., will be needed, and, in addition, consideration will have to be given to the possibility of storing respiratory samples.

Techniques of Storage

Unprocessed

Many biological samples may be stored entirely unprocessed with maintenance of practically all of their inorganic content. Unprocessed storage, however, is complicated by breakdown of organic material from bacterial and other enzyme action, and by loss of volatile gases. In addition, continued breakdown of organic material will cause a change in pH of body fluids, to the extent that, for example, estimation of pH must be made immediately on taking the sample to ensure reliability. Storage without processing is particularly applicable in the estimation of the inorganic content of urine, sweat, and feces, namely, sodium, potassium, chlorides, calcium, magnesium, manganese, bicarbonate, and zinc. Phosphates and pyrophosphates, however, decompose rapidly without special handling. Because of the strongly odoriferous contamination of organic breakdown, unprocessed storage is not recommended for more than a week or so.

Chemical Preservation

Various chemical preservatives have been utilized, particularly for the preservation of urine, and to some extent, feces. Acidification of urine in particular, has been found effective for preservation of the stability

of the 17 OH-ketosteroids, the catecholamines, and serotonin, in addition to the organic materials mentioned above. Boric acid (Frost and Richards, 1945)⁴ in a concentration of 1gm/100 ml, has been found useful for this purpose, while storage containers pre-dosed with benzoic acid were used successfully for urine collection in the Gemini VII 14-day mission. Storage of urine for catecholamine estimation, however, requires much stronger acidification, and for this purpose hydrochloric acid is used, in a concentration of 14 ml/liter. Cargille Laboratories, Inc., Cedar Grove, New Jersey, market preservative tablets used particularly for maintenance of urine specimens in transit. These are compound buffered acid tablets based on a formula proposed by Kingsbury (1925)¹¹, and will preserve specimens for at least 5 days. A similar type of tablet is marketed by Aloe Scientific, St. Louis, and is known as Brook Preservagent. The manufacturers claim that this reagent should preserve urine for a practically unlimited duration for "all conventional urine tests." It will be noted that strong acidification causes denaturation of proteins. Other preservatives have also been used in urine, including chloroform, formaldehyde, toluene, and thymol, particularly for the purpose of keeping down bacterial growth.

Potassium fluoride (Major, 1923)¹⁶ is commonly used for very prolonged periods as a preservative for urine and blood in the estimation of glucose, and has been used for 10 days in storage for estimations of blood urea, non-protein nitrogen, creatinine, cholesterol and uric acid (Henry, 1964)⁷. Antibiotics have also been used for prevention of bacterial growth in blood (Bayliss and Wooton, 1954)¹.

Other preservatives of interest include the use of citrate and EDTA in the preservation of uncoagulated blood for certain clotting factors and other studies; the addition of NaCl to serum for the preservation of complement for 2 to 5 days; and the use of intracellular and extracellular additives in freezing of blood cells. Freezing will be discussed separately.

Refrigeration

Refrigeration at temperatures of about 4° - 10° C (preferably the

former) provides a useful method of halting bacterial growth, and reducing organic change. With urine specimens, refrigeration is commonly combined with acidification. MacIntyre and Mou (1965)¹⁵ carried out a study to determine the persistence of leucocytes and erythrocytes in urine and showed that unrefrigerated, highly dilute, or markedly alkaline urine samples yield inaccurate counts within a few days. In refrigerated acid urines, however, counts are retained from 10-45 days. In another study, Ryan and Mills (1963)²² showed that bacteria will not multiply when the urine is kept below 10° C for four days. There is no reason to doubt that the organic constituents in urine, sweat, feces, and serum will remain stable for up to about 7 days when stored at temperatures of 4° C or below. With longer periods, it is probable that degradation will take place.

Freezing

For more prolonged maintenance of the organic content of the biological samples, freezing at temperatures down to -75° to -100° C becomes necessary. At this temperature, or below, almost all biological samples retain their organic or inorganic content at values comparable with those found at the time of acquisition. The final duration of this stability is unknown but it is considered to be well in excess of 45 days, and may even be years.

A study by Levey and Jennings (1950)¹² showed that with prolonged storage of pooled blood or plasma at -10° C, the total protein, albumin, chloride and urea remained stable, although there was loss of gamma globulin. Another study by Davison (1953)² indicated that the level of acid phosphatase remained stable after storage for 112 days at -15° C. Strumia et al., (1952)²³ found even longer periods of stability in plasma stored at -20° C with occasional rises to -15° C. They reported no apparent changes in albumin and globulin over a 10 year period, although some loss of prothrombin activity and variable loss of complement activity occurred after 5 years. A more comprehensive study for a shorter period was undertaken by Walford et al., (1956)²⁴ who found no change in albumin, globulin, total protein, urea, uric acid, creatinine, cholesterol, bilirubin, chloride, amylase, cholinesterase, and acid phosphatase

after six months storage at -10°C . Changes were observed, however, in non-protein nitrogen, glucose, and alkaline phosphatase. In this connection, Gomiori (1954)⁵ found significant losses in alkaline phosphatase content after storage for seven days in what he called the "frozen state."

A useful table on the storage stability of various materials in frozen plasma is appended below, from the paper of Walford et al., (1956)²⁴. The references in the table are not included in this text but may be obtained from Walford's paper. (See Table II).

Certain further comments are in order. Cells and platelets are destroyed by freezing; consequently the direct technique is inapplicable to the preservation of whole blood or body cells. As another limitation it must be remembered that serum bilirubin, even in the frozen state, breaks down readily when exposed to light. A further limitation is found in the storage of viruses from, for example, upper respiratory microbiological samples. The temperature of -20°C is not low enough for continued preservation of most viruses, and temperatures in the range of -60°C are required.

It would appear, however, that for most biochemical purposes, immediate freezing of the samples at -20°C , along with an acid preservative for urine, would meet storage requirements for 45 days or more.

As noted, whole blood cells and platelets are destroyed by freezing, even at minimal freezing temperatures. Several theories have been evoked to account for the mechanism of destruction. These are discussed by Luyet (1964)¹⁴ and by Richards et al., (1964)²⁰. Essentially there are three theories, namely, that destruction of cells occurs by rupture from ice crystals, by freeze dehydration, or by localized concentration of solutes following dehydration. The last named receives the greatest support.

Luyet, however, showed in 1949, that very thin films of blood could be rapid frozen in liquid nitrogen, and be recovered intact with rapid thawing. The development of protective additives, however, was required before practical use could be made of frozen blood. Various additives, and the techniques of their use, are discussed by Greaves et al., (1963)⁶, Huggins (1964)⁸, Huntsman et al., (1962)⁹, Huntsman et al., (1964)¹⁰, Meryman (1963)¹⁷,

TABLE II
Variation in Chemical Constituents of Frozen Plasma after
a 6-Month Interval of Storage

Substance	Mean Concentration		Per Cent Change	t Value*	Coefficient of Variation of Individual Determinations	Method
	Initial Value	Final value (6 mo. later)				
					per cent	
Albumin	3.48 Gm/100 ml	3.53	+1.4	1.12	5.4	Gornall et al. ¹³
Globulin	1.65 Gm/100 ml	1.70	+3.0	0.54	6.5	Gornall et al. ¹³
Total Protein	5.13 Gm/100 ml	5.23	+1.7	2.0	3.3	Gornall et al. ¹³
Urea	10.4 mg/100 ml	10.6	+2.6	1.1	7.9	Gentzkow ¹¹
Nonprotein nitrogen	26.3 mg/100 ml	23.3	-11.4	7.4	6.1	Daly ⁶
Uric acid	3.4 mg/100 ml	3.5	+3.2	1.53	9.6	Brown ⁴
Creatinine	0.55 mg/100 ml	0.54	-2.7	1.08	3.7	Folin and Wu ¹⁰
Cholesterol	160.8 mg/100 ml	161.1	+0.2	0.15	5.6	Sobel and Mayer ¹⁸
Glucose	353.0 mg/100 ml	334.0	-5.4	2.86	9.0	Hiller ¹⁶
Bilirubin	0.33 mg/100 ml	0.31	-5.2	1.43	15.5	Ducci and Watson ⁹
Chloride	68.8 mEq/l	70.0	+1.8	1.55	2.7	Van, Slyke and Hiller ²²
Amylase	33.6 units	32.8	-2.4	0.93	45.0	Somogyi ¹⁹
Cholinesterase	164.5 μ M/ml	170.3	+3.5	2.08	12	de la Huerga et al. ⁸
Alkaline phosphatase	1.24 Bodansky units	0.93	-25	3.38	41	Hawk et al. ¹⁴
Acid phosphatase	0.30 Bodansky units	0.22	-28	1.37	85	Hawk et al. ¹⁴

*With 18 degrees of freedom a ^t value greater than 2.10 would indicate significant change on a 5 per cent probability level.

Meryman (1964)¹⁸, Richards et al., (1964)²⁰, and Rinfret (1963)²¹, while the Proceedings of the Conference on Long Term Preservation of Blood Cells sponsored by the NAS and NRC in 1964 contains some 22 papers by various authorities, including Drs. Luyet, Rinfret, Richards, Strumia, Meryman, etc.

Essentially two types of additive and two types of procedure are used. Endocellular cryophylactic agents (ECA) such as glycerol, dimethylsulfoxide (DMSO) and propylene glycol, penetrate the cell, sequester the amount of water deposited as ice, and protect living cells by minimizing the degree to which dissolved solutes concentrate. Various sugars have also been used for the purpose but are not so effective. DMSO is reported as being superior to glycerol (Lovelock and Bishop, 1959)¹³. The use of glycerol type additives demands a slow controlled rate of freezing to about -100°C , although storage temperatures of -20°C have been employed for several weeks without excessive loss. On thawing, however, a momentary osmotic imbalance exists between the cell and surrounding fluid, which tends to cause lysis of the cell. Consequently the additive has to be removed before cell damage results.

Rapid freezing techniques, e.g., spraying blood into liquid nitrogen, were initiated by Meryman and Kafig in 1954¹⁹, using glucose as an additive without the requirement for washing. These rapid freeze techniques have been developed on a large scale by Rinfret of the Linde Company, using polyvinylpyrrolidone (PVP) as a non-penetrating extracellular additive. Rinfret (personal communication) points out, however, that the rapid freeze technique with PVP does not preserve white blood cells and platelets, whereas white blood cells and, to some extent, platelets, are preserved with controlled rate freezing, using glycerol or DMSO.

The final duration of stability in freezing conditions is unknown. As a consequence of freezing and thawing there is a loss of 2-3% free hemoglobin and about the same percentage of red cells. Communication with Dr. Rinfret indicates that white blood cell motility and phagocytic activity should be retained for at least 45 days, and during that period there should be 80% recovery of a differential white count. Reticulocytes may well be better preserved than adult red cells. Hematocrit may vary a little, depending upon any crenation or spherocytosis of red cells. A paper by Huntsman

et al., (1964)¹⁰ indicates that hemoglobins were retained in solutions prepared from normal red cells which had been stored at -196°C for two years.

Lyophilization

The technique of lyophilization, also known as vacuum freeze drying was initially developed for the storage of serum and plasma (Flosdorf and Mudd, 1935)³. The principles, logistics, and uses of the technique, are comprehensively discussed in the final report of the Biomedical Instrumentation Requirements for a Manned Orbiting Laboratory (1965) prepared by Beckman Instruments, Inc., for the U.S. Air Force. The process essentially involves three phases, namely, freezing, primary drying and secondary drying. Freezing techniques employed may be evaporative or pre-freezing. In the evaporative method, rapid cooling is accomplished by exposing a large surface area of the specimen to a rapid reduction in pressure. This process may be accompanied by excessive frothing of the material which can be controlled by previous degassing from exposure to an intermediate pressure, followed by sudden lowering of temperature, or by centrifuging the material during pressure reduction. If the material is pre-frozen before exposure to the reduced pressure no frothing will take place.

The primary drying is accomplished by maintaining the frozen specimen in a drying chamber with a pressure below that of the vapor pressure of water, and concurrently removing the water vapor evolved from the specimen in a cold trap or desiccant bed. This technique reduces the residual moisture content to about 4-6%. To effect the desired reduction to 1%, secondary drying is accomplished in a vacuum over phosphorus pentoxide.

The technique is applicable to plasma, serum, urine, feces, and various biological fluids, but, according to Meryman (1963)¹⁷, in practice only two mammalian cells can survive the process, namely, erythrocytes, and spermatozoa. Specimens impregnated with glycerol or DMSO cannot be freeze dried since these additives are not volatile. The technique is complex, the equipment is bulky and heavy, and the method does not seem to offer any advantages not possessed by low temperature freezing.

Drying

Drying, or desiccation, in the form of drum drying or spray drying, has been utilized in the food and pharmaceutical industries, and for biological materials. Strumia and associates (1952)²³ found that in plasma stored in the desiccated state, albumin, globulin, and prothrombin remained stable for about 5 years, although the stability of complement was variable. Desiccation, however, also requires complex equipment, and has little to offer over cryogenic freezing except the advantage of preservation at room temperature. Meryman (1963)¹⁷, in fact states; "But whereas one can store at liquid nitrogen temperature with complete confidence that no reactions will take place regardless of the instability of the specimen, in the dried state one can never have complete confidence that all possible deleterious reactions have been prevented. One cannot predict in advance that any given living cell which can be dried can be stored successfully."

Special Considerations

Certain tests do not lend themselves to storage of the materials involved. In particular, pH of urine or serum is extremely sensitive, and may change within hours or even minutes of taking the sample. Very rapid changes also occur in blood pO_2 and pCO_2 on exposure to any atmosphere, and it is not considered feasible to store blood for this measure. Clotting time, bleeding time, and clot retraction time must of course be measured on the spot.

With certain other measures there is considerable doubt as to whether they will be reliable. Platelets for example do not survive straight freezing. According to Rinfret there is some recovery of platelets with controlled rate cooling techniques and the use of suitable additives such as glycerol or DMSO. The extent of this recovery is not clear, although it is doubtful if meaningful platelet counts can be made. Useful qualitative, and perhaps even semi-quantitative, information on platelets can be obtained, however, from blood smears. Again, it is not clear how long unstained blood smears will last, although it is believed to be beyond 45 days. It has been suggested that smears might be fixed in alcohol before storage.

Tests of RBC mass and survival normally call for tagging of the cells with the radioisotope chromium-51, or di-isopropylfluorophosphate (DFP). There seems to be no reason why blood tagged in this manner should not store just as readily as untagged blood. The half-life of CR_{51} is 27 days and there will be little remaining at the end of a 45-day mission. DFP, however, should remain stable and undecayed throughout.

Although the storage should be no major problem, collection of material for studies in clotting dynamics may present difficulty. Many of the studies, and, in particular, the estimation of anti-hemophilic globulin, require for best results that the citrated blood be centrifuged at $4^{\circ}C$ for collection of a platelet poor plasma.

RISA has been used as a tagging material for the measurement of blood volume and its use has been suggested in space flight. It should be noted, however, that since the half-life of RISA is only 8 days, the use of the material will not be very practicable when prolonged storage is envisaged. A dye or other tagging method would be more appropriate.

With respect to blood ADH and ACTH, it is not known at this time how long the values remain stable in the unprocessed or refrigerated state. With freezing, however, stability is expected for periods greater than 45 days.

The requirement for karyotyping raises a problem. Normally, specimens for karyotyping are grown in tissue culture media for two to four days prior to harvesting and processing. The harvesting time is critical. Consequently for any mission longer than a few days direct storage is not possible. It is not known whether successful karyotyping can be done on frozen stored cells.

Storage of microbiological samples raises still another problem. It is necessary first to place the sample in broth before processing. Viruses can be stored for prolonged periods at $-60^{\circ}C$, and some at $-20^{\circ}C$, but most bacteria, including the common pathogens, do not withstand freezing at those temperatures, although somewhat surprisingly they can be stored by lyophilization. It may be that they will withstand very rapid freezing at cryogenic temperatures, but no information can be found on this matter.

For respiratory measures, storage of both inspiratory and expiratory

gases is required. Rubberized or polyvinyl plastic bags are not suitable for this purpose since in the course of time gas can diffuse through the material. Samples could, if necessary, be stored in metallic or glass containers in relatively small quantities, although gas volumes would have to be measured in orbit, using some system such as an integrating pneumotachograph. Considerable development would be required to prepare sampling techniques to determine the minimum size of sample required. Once the sample was stored, however, it would remain stable indefinitely.

Acceptable Duration of Storage

From the above considerations, Table III has been prepared to indicate the acceptable duration of storage under different circumstances for standard clinical laboratory techniques. It is emphasized that by no means all of the durations quoted have been validated experimentally, and it is recommended that tests be conducted to determine the reliability of the storage.

Most of the figures quoted with respect to biochemical stability in the refrigerated state are obtained from Henry's Clinical Chemistry (1964)⁷. In some cases, particularly for long duration storage, the actual facts are not fully known and numbers are quoted in the table on the basis of extrapolation, taking into account the biochemical relations of the materials under consideration.

The authorities contacted have been justifiably unwilling to place a definite time limit on a storage procedure, and the numbers quoted are probably conservative in many cases. For example, plasma or serum stored at -60° to -100° C probably retains its stability for months, if not years, but is noted in the table to hold its stability for >45 days.

Although the maximum duration of mission envisaged is in fact 90 days, the maximum duration of storage quoted in this table is only 45 days, since a 90-day mission is expected to have at least one re-supply. It is very probable however, that under deep freezing, with additives where required, all samples quoted as being storable for >45 days would in fact be storable within acceptable limits for >90 days.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration <5° C	Preservative & Refrigeration	Lyophilization	Freezing -75 to -100°C	Remarks
Urine							
Sodium	>45 days	Will not be dis- turbed by buffered acid preservative holding pH3-4.	Not required but hygienic	Not required	Not required	Not required	May be absorbed on to some plastic containers.
Potassium	"	"	"	"	"	"	"
Chlorides	"	"	"	"	"	"	"
Calcium	"	"	"	"	"	"	
Magnesium	"	"	"	"	"	"	
Manganese	"	"	"	"	"	"	
Bicarbonate	"	"	"	"	"	"	Can be stored unpro- cessed if no acid growth.
Nitrogen	"	"	"	"	"	"	
Zinc	"	"	"	"	"	"	
Sulphates	24 hours	"	4-7 days	n/a	>45 days	>45 days	
17 OH-ketosteroids	n/a (require acidification)	>45 days with buffered acid	n/a (require acidification)	Not required but hygienic	Not required	Not required	
Catecholamines	"	"	"	"	"	"	Acidify with HCL.
Aldosterone	"	"	"	"	"	"	
ADH	"	"	"	"	"	"	
ACTH	"	"	"	"	"	"	
Serotonin	"	"	"	"	"	"	
Proteins	Few days	n/a	1 - 2 weeks	n/a	>45 days	>45 days	
Glucose	Few hours	7 days & KF	Few days	>7 days & KF	"	"	Formaldehyde & CHCL ₃ also used as preservative

Continued on next page.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration <5° C	Preservative & Refrigeration	Freezing Lyophilization -75 to -100°C	Remarks
Urine (Continued)						
Phosphates	Few hours	Will not be disturbed by buffered acid preservative holding pH3-4	>7 days	>45 days & HCL	>45 days	
Pyrophosphates	"	"	"	"	"	
Mucoproteins	"	"	"	Denatured by HCL	"	
Creatine	Hours to days	Thymol used & no storage increase.	few days	1-4 weeks & petroleum.	"	Acid not applicable.
Creatinine	"	"	"	"	"	
Sp. gr.	>45 days	n/a	Not required but hygienic	n/a	"	
pH	Minutes	n/a	n/a	"	n/a	Storage not possible.
Serum						
Alkaline phosphatase	Hours - days	n/a	Few days	n/a	>45 days	>45 days
Sodium	>45 days	"	Not required	n/a	Not required	Not required.
Potassium	"	"	"	"	"	"
Chlorides	"	"	"	"	"	"
Phosphates	Few hours	"	1 - 2 weeks	"	>45 days	>45 days
Calcium	>45 days	"	Not Required	"	Not Required	Not required
Magnesium	"	"	"	"	"	"
Manganese	"	"	"	"	"	"
Bicarbonate	"	"	"	"	"	If no acid growth.

Continued on next page.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration & Preservative <5° C	Refrigeration & Preservative	Freezing Lyophilization -75 to -100°C	Remarks
Serum (Continued)						
Zinc	>45 days	n/a	Not required	n/a	Not required	Not required
Sulphates	24 hours	"	4 - 7 days	"	>45 days	>45 days
ADH	?	"	?	"	"	"
ACTH	?	"	?	"	"	"
NPN	1 day	"	Few days	n/a	>45 days	>45 days
BUN	"	"	"	"	"	"
Uric Acid	3 days	>3 days c NaF	3 - 5 days	"	"	"
Glucose	<24 hours	7 days c KF	"	>7 days c KF	"	"
						NaF, thymol also pre- servative.
Lipids	1 - 3 days	n/a	4 - 11 days	n/a	"	"
Proteins	3 days	"	>30 days	"	"	"
Mucoproteins (etc.)	2-7 days	"	>7 days	"	"	"
Amino acid nitrogen	<4 days	"	4 - 7 days	"	"	"
Total nitrogen	"	"	"	"	"	"
Creatine	Hours	"	1 day	"	"	"
Creatinine	Hours	"	1 day	"	"	"
Bilirubin	Minutes to hours	"	4 - 7 days	"	"	"
PBI	>45 days	"	>45 days	"	"	"
Thyroxin	Days	"	? weeks	"	"	"
TBPA	Days	"	? weeks	"	"	"
pH	Minutes	"	n/a	n/a	n/a	n/a
						Storage not feasible.
Sweat						
Sodium	>45 days	n/a	Not required but hygienic	n/a	Not required	Not required
Calcium	"	"	"	"	"	"

Continued on next page.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration <5° C	Preservative & Refrigeration	Freezing Lyophilization -75 to -100°C	Remarks
Sweat (Continued)						
Potassium	>45 days	n/a	Not required but hygienic	n/a	Not required	Not required
Chlorides	"	"	"	"	"	"
Phosphates	Hours	"	>7 days	"	> 45 days c̄ HCL	>45 days
Magnesium	> 45 days	"	Not required	"	Not required	Not required.
Bicarbonate	"	"	"	"	"	"
						Can be stored unpro- cessed if no acid growth.
Zinc	"	"	"	"	"	"
Nitrogen	"	"	"	"	"	"
Feces						
Sodium	> 45 days	n/a	Not required but hygienic	n/a	Not required	Not required
Potassium	"	"	"	"	"	"
Chlorides	"	"	"	"	"	"
Phosphates	Hours	"	>7 days	"	>45 days	>45 days
Calcium	> 45 days	"	Not required but hygienic	"	Not required	Not required
Nitrogen	"	"	"	"	"	"
Total lipids	>5 days	"	> 5 days	"	>45 days	>45 days
Plasma (tagged)						
Volume	>45 days	n/a	>45 days	"	>45 days	>45 days
Whole Blood						
Lactic Acid	Minutes	n/a	Hours	n/a	>45 days	>45 days
						Preservation assisted by KF.

Continued on next page.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration <5° C	Preservative & Refrigeration	Lyophilization	Freezing -75 to -100°C	Remarks
Whole Blood (Continued)							
RBC count	Not known	See "Remarks"	Probably >21 days	n/a	n/a	>45 days \bar{c} suitable addi- tives.	Specimens taken with citrate or EDTA anti- coagulant.
WBC count	Probably days	See "Remarks"	"	"	"	"	"
WBC motility and phagocytic activity	Probably hours	"	"	"	"	"	"
Platelet count	"	"	"	"	"	Doubtful	See Text
Hemoglobin	Probably days	"	"	"	"	>45 days \bar{c} suitable addi- tives.	
Hematocrit	Probably days	"	"	"	"	"	
Reticulocytes	"	"	"	"	"	"	
RBC mass	"	n/a	"	"	"	"	CR ₅₁ half-life 27 days
RBC survival	"	"	"	"	"	"	
Blood smear	Probably >45 days	>45 days \bar{c} alcohol fix.	n/a	"	"	n/a	
Clotting Dynamics							
Clotting time	Immediate	n/a	n/a	n/a	n/a	n/a	Storage not feasible.
Bleeding time	"	"	"	"	"	"	"
Clot retraction time	"	"	"	"	"	"	"
Platelet adhesiveness	"	"	"	"	"	Doubtful	See Text
PTC analysis	Not known	"	Probably days	"	>45 days	>45 days	Citrated specimens
Fibrinogen	Probably hours	"	"	"	"	"	"
Fibrinolytic activity	"	"	"	"	"	"	"
Prothrombin activity	"	"	"	"	"	"	"

Continued on next page.

TABLE III ACCEPTABLE DURATION OF STORAGE

Measure	Unprocessed	Preservative	Refrigeration & Preservative <5° C	Refrigeration	Lyophilization	Freezing -75 to -100°C	Remarks
Clotting Dynamics (Continued)							
Anti-hemophilic globulin	Probably hours	n/a	Probably days	n/a	>45 days	>45 days	Citrated specimens
Immune studies							
Immunoglobulin	About 24 hours	n/a	2 - 5 days	n/a	n/a	>45 days	Serum specimen
Complement	"	3-4 days ± NaCl	"	"	"	"	"
Antibodies	"	n/a	1 - 2 days	"	"	"	"
Cytogenetic studies							
Slides (Karyotyping)	n/a	2-4 days in culture	n/a	n/a	n/a	Not known	May be possible after freezing, if cells viable
Miscellaneous							
Respiratory quotient	n/a	n/a	n/a	n/a	n/a	n/a	Storage possible with special equipment.
Metabolic rate	"	"	"	"	"	"	See Text.
Blood pO ₂	Immediate	"	"	"	"	"	Storage not feasible.
Blood pCO ₂	"	"	"	"	"	"	Storage not feasible.
Bacterial specimen	n/a	"	"	"	>45 days	"	Destroyed by freezing.
Viral specimens	"	"	"	"	n/a	>45 days.	

From the above table it will be observed that freezing of samples at -75° to -100° C, with or without additives for the protection of cells, meets practically all requirements of storage for those tests in which storage is possible. For all the required biochemical testing, freezing at temperatures of about -20° C would appear to meet the requirements. Lyophilization, with the exception of storage of bacterial samples, seems to serve no purpose that could not be as well served by freezing, with less equipment. In addition, desiccation has little if any advantage and does not appear to be reliable as a stable technique.

Refrigeration, at standard laboratory temperatures of around $4-5^{\circ}$ C, would seem to be useful for holding samples stable for about a week. The stability of materials under refrigeration for more prolonged periods seems to depend more on an accompanying acidification, or a preserving agent, than on the refrigeration. A major advantage of refrigeration, however, is the reduction in odoriferous organic breakdown of the samples.

Preservatives, excluding anti-coagulants, have least usefulness in serum and blood, and most in the urine, where the most generally useful preservative is acidification. The latter would seem to be best applied in the form of a compound buffered acid tablet, or pre-dosed container. Some materials, however, e. g., catecholamines, demand much stronger acidification.

The storage of unprocessed material is particularly applicable in the testing for inorganic constituents of biological samples, although, if storage is needed for more than a few days, refrigeration of the samples is recommended in addition, for hygienic purposes.

The minimum storage requirement for different flight durations, based on the above, is shown in Table IV. Symbols used in the table are as follows:

- U: unprocessed
- P: preservative
- R: refrigeration
- F: freezing
- L: lyophilization

TABLE IV

MINIMUM STORAGE REQUIREMENT

MEASURE	15 Days	30 Days	45 Days
<u>Urine</u>			
Sodium	U	U + R	U + R
Potassium	U	U + R	U + R
Chlorides	U	U + R	U + R
Calcium	U	U + R	U + R
Magnesium	U	U + R	U + R
Manganese	U	U + R	U + R
Bicarbonate	U	U + R	U + R
Nitrogen	U	U + R	U + R
Zinc	U	U + R	U + R
Sulfates	F	F	F
17 OH-ketosteroids	P	P + R	P + R
Catecholamines	P	P + R	P + R
Aldosterone	P	P + R	P + R
ADH	P	P + R	P + R
ACTH	P	P + R	P + R
Serotonin	P	P + R	P + R
Proteins	R	F	F
Glucose	P + R	F	F
Phosphates	P + R	P + R	P + R
Pyrophosphates	P + R	P + R	P + R
Mucoproteins	F	F	F
Creatine	F	F	F
Creatinine	F	F	F
Specific gravity	R	R	R
pH	n/a	n/a	n/a

MEASURE	15 Days	30 Days	45 Days
<u>Serum</u>			
Alkaline phosphatase	F	F	F
Sodium	U	U	U
Potassium	U	U	U
Chlorides	U	U	U
Phosphates	R	F	F
Calcium	U	U	U
Magnesium	U	U	U
Manganese	U	U	U
Bicarbonate	U	U	U
Zinc	U	U	U
Sulfates	F	F	F
ADH	?F	F	F
ACTH	?F	F	F
NPN	F	F	F
BUN	F	F	F
Uric acid	F	F	F
Glucose	P + R	F	F
Lipids	F	F	F
Proteins	R	R	F
Mucoproteins (etc.)	F	F	F
Amino acid nitrogen	F	F	F
Total nitrogen	F	F	F
Creatine	F	F	F
Creatinine	F	F	F
Bilirubin	F	F	F
PBI	U	U	U
Thyroxine	?R	?R	?F
TBPA	?R	?R	?F
pH	n/a	n/a	n/a

MEASURE	15 Days	30 Days	45 Days
<u>Sweat</u>			
Sodium	U	U + R	U + R
Calcium	U	U + R	U + R
Potassium	U	U + R	U + R
Chlorides	U	U + R	U + R
Phosphates	R	F	F
Magnesium	U	U + R	U + R
Bicarbonate	U	U + R	U + R
Zinc	U	U + R	U + R
Nitrogen	U	U + R	U + R
<u>Feces</u>			
Sodium	U	U + R	U + R
Potassium	U	U + R	U + R
Chlorides	U	U + R	U + R
Phosphates	R	F	F
Calcium	U	U + R	U + R
Nitrogen	U	U + R	U + R
Lipids	F	F	F
<u>Plasma</u>			
Tagged	U	U + R	U + R
PTC analysis	?R, or F	F	F
Fibrinogen	?R, or F	F	F
Fibrinolytic activity	?R, or F	F	F
Prothrombin activity	?R, or F	F	F
Anti-hem. glob.	?R, or F	F	F

MEASURE	15 Days	30 Days	45 Days
<u>Whole Blood</u>			
Lactic acid	F	F	F
RBC count	R	F	F
WBC count	F	F	F
WBC motility, etc.	F	F	F
Platelets	F or n/a	F or n/a	F or n/a
Hemoglobin	R	F	F
Hematocrit	R	F	F
Reticulocytes	R	F	F
RBC mass	R	F	F
RBC survival	R	F	F
Smear	U	U	?U + P
Clotting time	n/a	n/a	n/a
Bleeding time	n/a	n/a	n/a
Clot retraction	n/a	n/a	n/a
Platelet adhesiveness	F or n/a	F or n/a	F or n/a
<u>Immune studies</u>			
Immunoglobulin	F	F	F
Complement	F	F	F
Antibodies	F	F	F
<u>Cytogenic studies</u>			
Karyotyping	F or n/a	F or n/a	F or n/a
<u>Miscellaneous</u>			
Blood pO ₂	n/a	n/a	n/a
Blood pCO ₂	n/a	n/a	n/a
Bacterial specimen	L	L	L
Viral specimen	F	F	F

On examination of the above table, it becomes obvious that if all the noted tests are required and each class of material is provided with its minimal storage needs, then even for the 15-day mission all forms of storage will still be required, namely, unprocessed, preserved, refrigerated, and frozen. This complexity would lead to problems of logistics, and a trade-off study is needed to determine the requirements in terms of weight, volume, power, etc., and complexity, for different types of storage. It would seem that requirements would be met by providing refrigerated storage for all materials, preserved or unprocessed, that do not require freezing, even if refrigeration is not necessary, and a low temperature (-75 to -100° C) facility for those that do, even if the freezing requirements of some would be met by temperatures of around -10° to -20° C.

At the same time, it is again emphasized that the storage techniques discussed have not been validated in all cases. It is recommended that the various techniques and durations of stable storage be experimentally tested under realistic conditions.

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